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A New Gliding Frog of the Genus *Rhacophorus* from Borneo

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Abstract: A rhacophorid frog from Borneo is divergent genetically and morphologically from Javanese *R. reinwardtii*, with which it was formerly confused, and is recognized as a distinct species. The frog differs from *R. reinwardtii* by an immaculate green dorsum and a black posterior thigh surface, which is studded with sky blue spots in the female. Because the frog is also divergent genetically and morphologically from the other congeners recently split from *R. reinwardtii*, we describe it as a new species, *R. borneensis*.

Key words: New species; MtDNA phylogeny; *Rhacophorus reinwardtii*; Borneo; Taxonomy

INTRODUCTION

The frog genus *Rhacophorus* Kuhl and Van Hasselt, 1822 occurs in Asia from India, Japan, the Philippines and China to Sulawesi (Frost, 2013). Discoveries and new descriptions of *Rhacophorus* species have rapidly increased in recent years, with the number of nominal species increasing from 57 (Frost, 1985) to 82 (Frost, 2013) in these three decades. The bulk of these new discoveries arose from morphological examination of samples from newly explored regions (e.g., Harvey et al., 2002; Wilkinson et al., 2005; Matsui and Panha, 2006; Bordoloi et al., 2007), but use of recently developed molecular techniques also prompted the discoveries (e.g.,

Ohler and Delorme, 2006) as in many other frogs (e.g., Stuart et al., 2006; Inger et al., 2008; Shimada et al., 2011).

Along with such increase in the number of species, changes in generic allocation of some *Rhacophorus* species have also resulted from molecular phylogenetic studies (e.g., Hertwig et al., 2012; Yu et al., 2013). By contrast, the generic status of some species related to *R. reinwardtii* (Schlegel, 1840) is unchanged since *R. reinwardtii* represents the type species of the genus (Ohler and Dubois, 2006). However, it has recently been recognized that *R. reinwardtii* itself contains in fact clusters of similar species (Ohler and Delorme, 2006; Chan and Grismer, 2010). Most recently, an additional new species was described from Vietnam (Rowley et al., 2012). Through these studies, *R. reinwardtii* has become a morphologically more uniform and geographically more narrowly ranging species, confined to

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FIG. 1. Male holotype (BORNEENSIS 22410) and allotype (BORNEENSIS 22411) of *Rhacophorus borneensis* in amplexus.

Borneo and Java (Chan and Grismer, 2010).

However, the range of *R. reinwardtii* is now further narrowed. During a joint expedition of the UMS (University Malaysia Sabah) and JICA (Japan International Cooperation Agency) to the Maliau Basin Conservation Area, Sabah, Malaysian Borneo, we collected a pair of specimens identified as *R. reinwardtii* (Fig. 1), but subsequent genetic analysis proved significant divergence of the specimens from topotypic *R. reinwardtii* from Java. Because the specimens are also morphologically distinguishable from Javanese *R. reinwardtii*, we describe them as a new species.

MATERIALS AND METHODS

DNA sequence data were obtained from tissues preserved in 99% ethanol for specimens of *Rhacophorus* sp. from Sabah (BORNEENSIS [BORNEENSIS collection, Institute for Tropical Biology and Conservation, University Malaysia Sabah] 22410, 22411), *R. kio* Ohler and Delorme, 2006 from Xuan Lien, Vietnam (KUHE [Kyoto University, Graduate School of Human and Environmental Studies] 55165–55170), and *R. nigropalmatus* Boulenger, 1895 from Sarawak, Malaysian Borneo (KUHE 53935). We used the same methods

for DNA extraction, and amplification and sequencing of the mtDNA fragments as those reported by Matsui et al. (2011) and Shimada et al. (2011). The resultant sequences (2406–2410 base pairs [bp] of partial sequences of mitochondrial 12S and 16S rRNA genes and the intervening tRNA gene) were deposited in GenBank (Accession numbers AB781693–781701). In addition to our own data, we used GenBank data of the 16S rRNA gene of *R. reinwardtii* (GQ204720, Java; AF458146, China; AF285225, Vietnam?; JN377364–377366, Sarawak; GQ204713, Malaysia), *R. norhayatii* Chan and Grismer, 2010 (JX219443, Malaysia; AY880527, locality unknown; AB728191, Endau Rompin, Peninsular Malaysia), *R. kio* (EU215532, EF564570, EF564571, EF646371, EF646372, all Yunnan, China), and *R. helenae* Rowley, Tran, Hoang, and Le, 2012 (JQ288087–288091, Vietnam) for tree construction and calculation of genetic distances (uncorrected p-distance).

Specimens examined are stored in BORN-EENSIS Collection, University Malaysia Sabah (BORNEENSIS) and Graduate School of Human and Environmental Studies, Kyoto University (KUHE). We took the following 24 body measurements to the nearest 0.1 mm with dial calipers, following Matsui (1984) and Matsui (1994): (1) snout-vent length (SVL); (2) head length (HL); (3) snout length (SL); (4) eye length (EL); (5) eye diameter (ED), diameter of the exposed portion of the eyeball; (6) tympanum-eye length (T-EL); (7) tympanum diameter (TD); (8) head width (HW); (9) internarial distance (IND); (10) interorbital distance (IOD); (11) upper eyelid width (UEW); (12) forelimb length (FLL); (13) lower arm and hand length (LAL); (14) first finger length (1FL), measured from distal edge of inner palmar tubercle; (15) inner palmar tubercle length (IPTL); (16) hindlimb length (HLL); (17) thigh length (THIGH); (18) tibia length (TL); (19) foot length (FL); (20) first toe length (1TOEL); (21) inner metatarsal tubercle length (IMTL); (22) third finger disk diameter (3FDW); (23) fourth finger disk diameter (4FDW); and (24) fourth toe disk diameter (4TDW).

SYSTEMATICS

In the phylogenetic tree constructed from a very short sequence (507 bp) of 16S (Fig. 2), all ingroup samples, except for *R. reinwardtii* probably from Vietnam (AF285225), formed a well supported clade (MLBS=95%,

BPP=1.00) including three groups whose relationships were unresolved. The first group formed a clade (MLBS=90%, BPP=0.99) and contained *Rhacophorus* sp. from Sabah, *R. reinwardtii* from an unknown locality of Malaysia (GQ204713) and Sarawak (JN377364–377366). The second group,

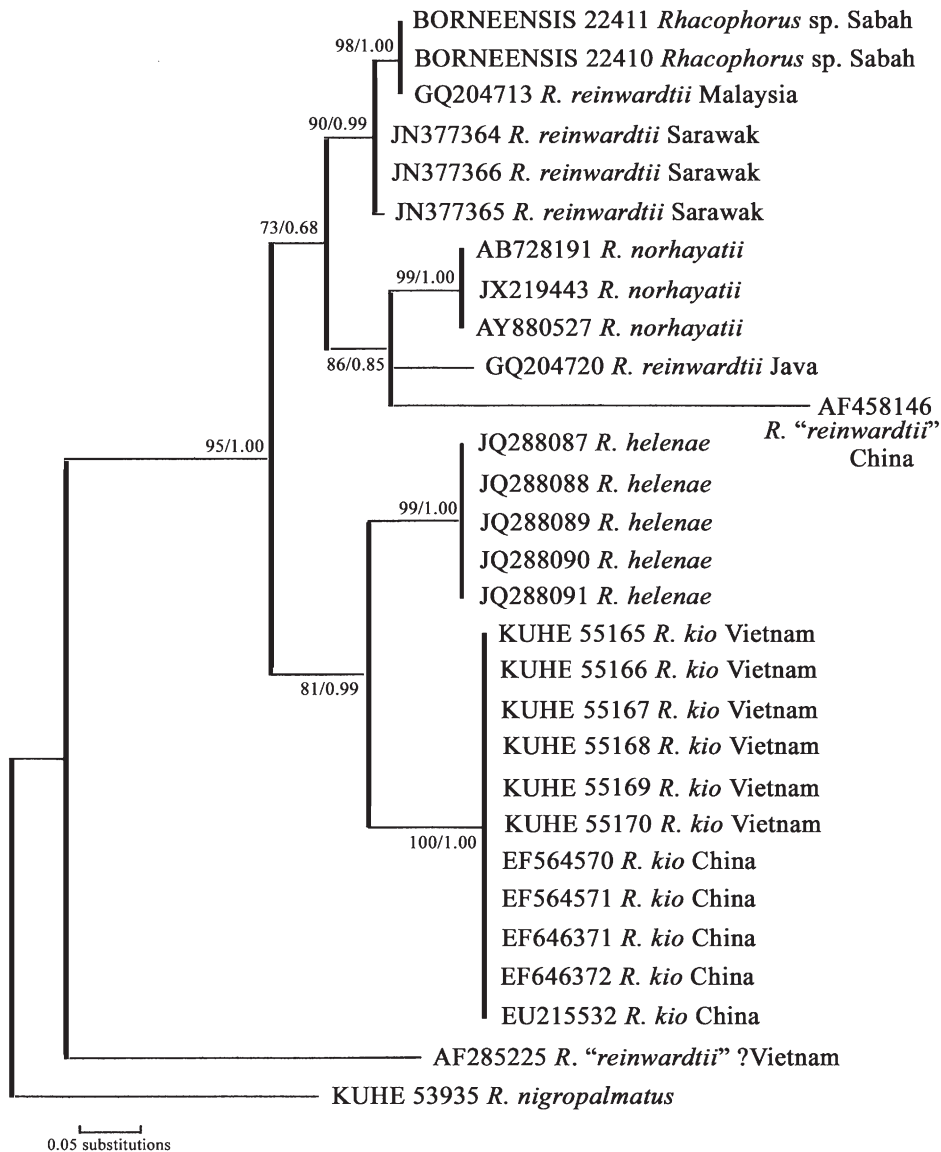


FIG. 2. ML tree from a 507 bp sequence of mitochondrial 16S rRNA gene for members of the *Rhacophorus reinwardtii* group. Numbers above or below branches represent bootstrap supports for ML inferences and Bayesian posterior probabilities (ML-BS/BPP).

TABLE 1. Uncorrected p-distances (in %) for short fragment of 16S rRNA among *Rhacophorus reinwardtii* and related species.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 <i>R. nigropalmatus</i> KUHE 53935																
2 <i>R. "reinwardtii"</i> AF285225	10.6															
3 <i>R. "reinwardtii"</i> AF458146	12.0	12.2														
4 <i>R. reinwardtii</i> Java GQ204720	10.3	9.7	8.2													
5 <i>R. norhayatii</i> AB728191	10.3	9.5	8.6	4.2												
6 <i>R. norhayatii</i> JX219443	10.3	9.5	8.6	4.2	0											
7 <i>R. norhayatii</i> AY880527	10.3	9.5	8.6	4.2	0	0										
8 <i>Rhacophorus</i> sp. Sabah BORNEENSIS 22410	10.5	9.5	9.8	5.2	4.2	4.2	4.2									
9 <i>Rhacophorus</i> sp. Sabah BORNEENSIS 22411	10.5	9.5	9.6	5.0	4.0	4.0	4.0	0.2								
10 <i>R. reinwardtii</i> Malaysia GQ204713	10.5	9.5	9.6	5.0	4.0	4.0	4.0	0.2	0							
11 <i>R. reinwardtii</i> Sarawak JN377365	10.1	9.6	9.2	4.6	4.0	4.0	4.0	1.2	1.0	1.0						
12 <i>R. reinwardtii</i> Sarawak JN377364, 366	10.1	9.5	9.2	4.6	4.0	4.0	4.0	1.0	0.8	0.8	0.2					
13 <i>R. helenae</i> JQ288087-91	10.2	10.5	11.0	6.3	7.3	7.3	7.3	5.4	5.4	5.4	4.6	4.6				
14 <i>R. kio</i> China EU215532	10.9	10.1	11.2	7.7	6.3	6.3	6.3	5.4	5.2	5.2	4.8	4.8	4.6			
15 <i>R. kio</i> China EF564570-71	10.9	10.1	11.2	7.7	6.3	6.3	6.3	5.4	5.2	5.2	4.8	4.8	4.6	0		
16 <i>R. kio</i> China EF646371-72	10.9	10.1	11.2	7.7	6.3	6.3	6.3	5.4	5.2	5.2	4.8	4.8	4.6	0	0	
17 <i>R. kio</i> Vietnam KUHE 55165-70	10.9	10.1	11.2	7.7	6.3	6.3	6.3	5.4	5.2	5.2	4.8	4.8	4.6	0	0	0

containing *R. norhayatii*, *R. reinwardtii* from Java (GQ204720), and *R. reinwardtii* from China (AF458146) was monophyletic in an ML tree (MLBS=86%), but support in a Bayesian tree was insufficient (BPP=0.85). The third group was monophyletic (MLBS=100%, BPP=0.99), and contained two clades (*R. kio* and *R. helenae*).

In the first clade, two sequences of *Rhacophorus* sp. from Sabah either differed very slightly (uncorrected p-distance in 16S rRNA of 0.2: Table 1) from or were identical with the sequence of *R. reinwardtii* from an unknown locality of Malaysia (GQ204713). *Rhacophorus* sp. from Sabah also differed from *R. reinwardtii* from Sarawak (JN377364–377366) with small distances (0.8–1.2%). By contrast, from the other taxa, the Sabah specimens substantially differed genetically by large genetic distances: p-distance of 4.0–4.2% from *R. norhayatii*, 5.0–5.2% from *R. reinwardtii* from Java, 5.2–5.4% from *R. kio*, 5.4% from *R. helenae*, and 10.5% from *R. nigropalmatus*. The distance between the Sabah specimens and *R. reinwardtii* from Java (5.0–5.2%) exceeded that between *R. norhayatii* and *R. reinwardtii* from Java (4.2%) or between *R. kio* and *R. helenae* (4.6%). Furthermore, the specimens of *Rhacophorus* sp. from Sabah are also separated morphologically from five nominal species (*R. reinwardtii* from Java, *R. norhayatii*, *R. kio*, *R. helenae*, and *R. nigropalmatus*) in congruence with genetic separation. Thus, we conclude that the specimen of *Rhacophorus* sp. from Sabah, as well as *R. reinwardtii* from Sarawak, Borneo is a distinct species and describe it as follows:

***Rhacophorus borneensis* sp. nov.**

Bornean Smaller Gliding Frog

Figs. 3–6

Rhacophorus reinwardtii Inger and Stuebing, 1997: 194 (part); Chan and Grismer, 2010: 43 (part).

Etymology

The specific name refers to the island of

Borneo, where the new species was found.

Holotype

Adult male (BORNEENSIS 22410), collected by Tomohiko Shimada between 20:00 and 23:00 h on 9 March 2005 from Camel Trophy field station (4°7482' N, 116°8886' E; ca. 1050 m asl.) of the Maliau Basin Conservation Area, Sandakan Division, Sabah, East Malaysia.

Paratype

An adult female (BORNEENSIS 22411) collected in amplexus with the holotype.

Diagnosis

The new species is assigned to the genus *Rhacophorus* by: frontoparietal lacking parieto-squamosal arch; distal end of terminal phalanx Y-shaped; intercalary cartilage present between terminal and penultimate phalanges of digits; tips of digits expanded into large discs bearing circum-marginal grooves; fingers webbed; vomerine teeth present (Inger, 1966; Liem, 1970; Brown and Alcala, 1994; Matsui and Panha, 2006), and to the *R. reinwardtii* group (Dubois, 1987). *Rhacophorus borneensis* can be distinguished from all other species of the *R. reinwardtii* group by the following combination of characteristics: male SVL 50.9 mm, female SVL 62.0 mm; solid green dorsum without any spots or markings; a yellowish orange venter with black spots; black axillary patch followed by dark spots on flanks; posterior surface of thigh yellowish orange in male and black, studded with sky blue spots in female; eye with white sclera; black webbing with blue veins and yellowish orange margins; a well-developed, double-lobed supracloacal dermal ridge.

Description of holotype

SVL 50.9 mm; body dorsoventrally compressed; head about as long (38.9%SVL) as broad (37.5%SVL); snout (18.1%SVL) longer than eye (14.1%SVL), rounded dorsally and sloping anteroventrally in profile, slightly pointed at tip and projecting slightly over

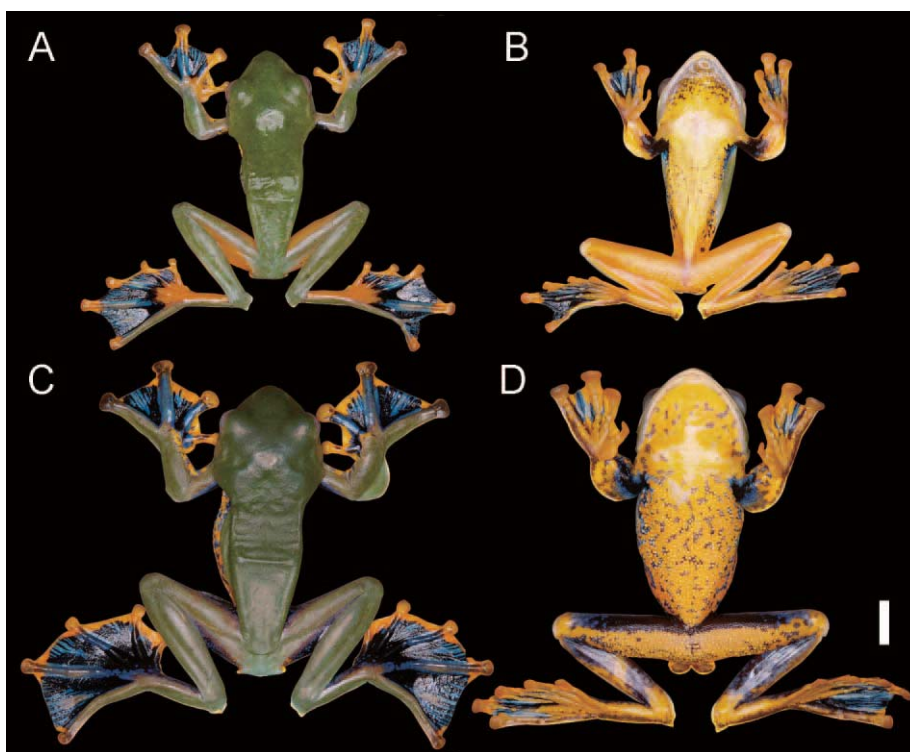


FIG. 3. Dorsal (A, C) and ventral (B, D) views of male holotype (BORNEENSIS 22410; A, B) and female paratype (BORNEENSIS 22411; C, D) of *Rhacophorus borneensis* in an anesthetized condition. Scale bar=10 mm.



FIG. 4. Lateral views of male holotype (BORNEENSIS 22410; A) and female paratype (BORNEENSIS 22411; B) of *Rhacophorus borneensis* in an anesthetized condition. Scale bar=10 mm.

lower jaw; canthus distinct, blunt; lore slightly oblique, slightly concave; nostril slightly protuberant, nearer to tip of snout than to eye; internarial distance (12.0%SVL) equal to

interorbital (12.0%SVL); latter wider than eyelid (10.0%SVL); pineal spot absent; eye large, protuberant, diameter (12.0%SVL) larger than eye-nostril; pupil horizontal; tympanum distinct, subcircular, length (7.7%SVL) about two-thirds eye diameter and separated from eye by one-fifth of tympanum diameter (1.6%SVL); vomerine teeth in nearly horizontal groups nearly touching anterior corners of choanae, groups separated by about half length of one group; a longitudinal opening into median subgular vocal sac at base of each side of tongue; tongue deeply notched posteriorly.

Forelimb moderately long (47.2%SVL); relative finger length $I < II < IV < III$; length of first finger (10.6%SVL) shorter than diameter of eye; tips of all fingers dilated into horizontally elongate large disks with circummarginal and transverse ventral grooves; disk of third

and fourth fingers (4.7% and 4.9%SVL) narrower than tympanum; broad web on all fingers, finger webbing formula I $1\frac{1}{2}$ – $1\frac{1}{2}$ II 0–0 III 0–0 IV (Fig. 5A); subarticular tubercle rounded, formula 1, 1, 2, 2; other indistinct subarticular tubercles present; supernumerary tubercles on metacarpals absent; prepollex prominent, oval; flat, indistinct inner (7.7%SVL) and indistinct, round outer palmar tubercles; indistinct nuptial pad on outer margins of prepollex and finger I.

Hindlimb long (154.4%SVL), about 2.6 times length of forelimb; thigh (49.0%SVL) slightly shorter than tibia (51.1%SVL), heels slightly overlapping when limbs are held at right angles to body; tibiotarsal articulation of adpressed limb reaching point between eye and nostril; foot (47.2%SVL) shorter than tibia; relative length of toes I < II < III < V < IV; tips of toes expanded into round disks with distinct circummarginal grooves, smaller than those of fingers (disk diameter of fourth toe 4.5%SVL); all toes webbed to disks, toe webbing formula I 0–0 II 0–0 III 0–0 IV 0–0 V (Fig. 5C); subarticular tubercles distinct,

rounded, formula 1, 1, 2, 3, 2; supernumerary tubercles absent; low, oval inner metatarsal tubercle, length (3.5%SVL) less than one-thirds length of first toe (12.0%SVL), but no outer metatarsal tubercle.

Dorsum smooth, free of skull; an oblique supratympanic fold from eye above tympanum, ending at above arm insertion; skin slightly loose in gular region; flanks roughly wrinkled; underside of chin and chest smooth, abdomen and thigh coarsely granular; dorsolateral fold absent; a wide, smooth-edged fringe of skin from outer edge of fourth finger to elbow; hindlimb with narrow fringes of skin along inner edge of first toe and outer edge of fifth, the latter continuing along tarsus to tibiotarsal articulation; dermal appendages at tibiotarsal articulation squarish, and vent bilobed.

Color

In life dorsum uniformly green, immaculate (Fig. 3A); limbs without crossbars; lips unpatterned white; ventral surface of lower arm through axilla to anterior half of flank inky black, speckled with fine yellow and blue spots (Fig. 4A); posterior flank yellowish orange flecked with black; anterior and posterior surfaces of thigh immaculate yellowish orange (Fig. 6A); dermal appendages on arm and leg including tibiotarsal projection and supraclacal dermal ridge lined with white; ventral surfaces of chest, belly, upper arm, thigh, tibia,

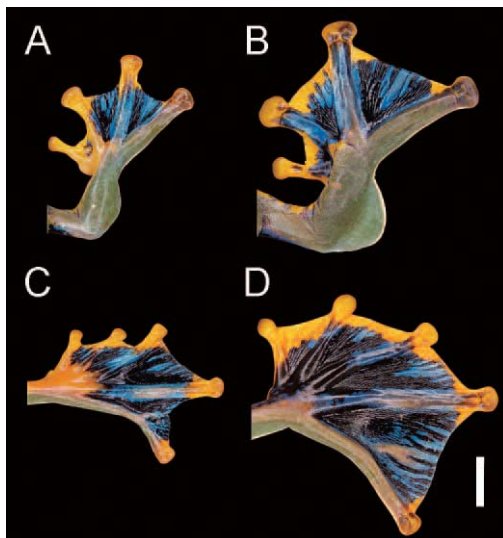


FIG. 5. Dorsal views of right hand (A, B) and foot (C, D) of male holotype (BORNEENSIS 22410; A, C) and female paratype (BORNEENSIS 22411; B, D) of *Rhacophorus borneensis* in an anesthetized condition. Scale bar=10 mm.



FIG. 6. Dorsal views of thigh of male holotype (BORNEENSIS 22410; A) and female paratype (BORNEENSIS 22411; B) of *Rhacophorus borneensis* in an anesthetized condition. Scale bar=10 mm.

and tarsus immaculate yellowish orange (Fig. 3B); chin mottled with small, black spots; dorsal surface of webbing between fingers II and IV black with fine blue streaks (Fig. 5A); webbing between fingers I and II yellowish orange with black spots; fingers I and II and discs yellowish orange; dorsal surface of webbing on toes black with fine blue streaks (Fig. 5C); web pigmentation entirely covers dorsal surfaces of toes except for base and distal edges, toe I, and toe discs which are yellowish orange; eye with black periphery and white sclera, iris dark red with enlarged reticulation in the dark (Fig. 1), but pale yellowish silver with a thin network of coppery brown reticulations under bright conditions (Fig. 4A).

In preservative, green fading to violet and yellowish orange fading to white, but black pigments unchanged.

Variation

Based upon the type series, the new species seems to be sexually dimorphic with the female being larger than the male (Table 2). The female has shorter head (34.7%SVL vs. 38.9%SVL in the male) and snout (15.2%SVL vs. 18.1%SVL), smaller eye (12.6%SVL vs. 14.1%SVL), and longer hindlimb (161.8%SVL vs. 154.4%SVL) than the male, all relative to SVL, but whether or not these represent sexual difference is undetermined due to small sample size. Both sexes are nearly uniform in coloration and pattern except for the ventral dark spots that are more developed in the female (Fig. 3D), and more drastically, the color of the thigh (Fig. 6); except for dorsal green, the thigh of the male is yellowish orange, but the anterior and posterior surfaces are black, studded with sky blue spots in the female. Underside of tibia is also largely covered by black in the female. The extent of black markings on webbing of hand and foot are more developed also in the female than the male (Fig. 5), and the black color covering the base of foot continues to inside of tarsus. By contrast, proximal margin of toe webbing and inside of tarsus and tibia are orange yellow in the male. Slightly loose skin in the gular

region of the male holotype is not seen in the female paratype.

Comparisons

Among members of *Rhacophorus*, *R. borneensis* superficially most resembles *R. reinwardtii* from Java, and to a lesser degree *R. norhayatii* from peninsular Thailand and Malaysia and *R. kio* and *R. helenae* from Indochina. Also, possibly sympatric *R. nigropalmatus* can be confused with *R. borneensis*. However, *R. borneensis* differs from them in some morphological characteristics.

From *R. nigropalmatus*, *R. borneensis* differs by much smaller body size (male SVL

TABLE 2. Measurements (in mm) of types of *Rhacophorus borneensis*. See text for abbreviations.

Sex	BORNEENSIS 22410 MA	BORNEENSIS 22411 FA
SVL	50.9	62.0
HL	19.8	21.5
HW	19.1	23.0
IND	6.1	6.7
IOD	6.1	7.1
UEW	5.1	5.6
SL	9.2	9.4
EL	7.2	7.8
ED	6.1	6.8
TD	3.9	4.4
E-TL	0.8	1.0
FLL	30.6	36.2
LAL	25.4	30.9
1FL	5.4	6.7
IPTL	3.9	4.4
THIGH	25.0	30.6
TL	26.0	31.8
FL	24.0	30.6
HLL	78.6	100.3
IMTL	1.8	2.7
1TOEL	6.1	8.6
3FD	2.4	4.0
4FD	2.5	3.8
4TD	2.3	3.0

50.9 mm; female SVL 62.0 mm vs. 78.7–88.6 mm and 89.0–100.3 mm, respectively, in *R. nigropalmatus* [Inger, 1966]), immaculate green dorsum (vs. green dorsum spotted with black in *R. nigropalmatus*), yellowish orange venter with black spots (vs. white venter with yellow mottling in *R. nigropalmatus*), black axillary patch (vs. absent in *R. nigropalmatus*), yellowish orange posterior surface of thigh in male, and black thigh, studded with sky blue spots, in female (vs. solid yellow in *R. nigropalmatus*), and incomplete webbing between fingers 1 and 2 (vs. fully webbed to disk in *R. nigropalmatus*).

Rhacophorus borneensis, with a male SVL of 50.9 mm and female SVL of 62.0 mm and double-lobed supracloacal dermal ridge closely resembles *R. reinwardtii* (male SVL 41.6–52.5 mm and female SVL 55.4–79.6 mm [Chan and Grismer, 2010]), but differs from it by immaculate green dorsum (vs. darkly spotted dorsum in *R. reinwardtii*) and posterior surface of thigh black, studded with sky blue spots in female (vs. yellow or orange in *R. reinwardtii*).

Rhacophorus borneensis is similar in male body size with *R. norhayatii* (SVL 50.9 mm vs. 41.7–64.7 mm in *R. norhayatii* [Chan and Grismer, 2010]) but differs from it by much smaller female body size (SVL 62.0 mm vs. 75.7–83.0 mm in *R. norhayatii* [Chan and Grismer, 2010]), having yellowish orange venter with black spots (vs. white venter with black marbling and blue mottling in *R. norhayatii*), and yellowish orange posterior surface of thigh in male (vs. black thigh, studded with sky blue spots in *R. norhayatii*).

Rhacophorus borneensis with a well-developed, double-lobed supracloacal dermal ridge resembles *R. kio*, but differs from it by having smaller body size (male SVL 50.9 mm and female SVL 62.0 mm) than *R. kio* (58.0–79.1 mm and 82.6–88.9 mm, respectively [Ohler and Delorme, 2006; Rowley et al., 2012]), yellowish orange venter with black spots (vs. bright yellow or lemon yellow venter in *R. kio*), posterior surface of thigh yellowish orange in male and black, studded with sky blue spots in

female (vs. solid yellowish orange in *R. kio*), eye with white sclera (vs. sclera yellow in *R. kio*), and black webbing with blue veins and yellowish orange margins (vs. extensive bright yellow to orange webbing in *R. kio*).

Finally, *R. borneensis* (male SVL 50.9 mm; female SVL 62.0 mm) is smaller than *R. helenae* (72.3–85.5 mm and 89.4–90.7 mm, respectively [Rowley et al., 2012]), and differs from it by having well-developed, double-lobed supracloacal dermal ridge (vs. supracloacal dermal ridge low and single-lobed in *R. helenae*), yellowish orange venter with black spots (vs. venter white in *R. helenae*), posterior surface of thigh yellowish orange in male and black, studded with sky blue spots in female (vs. bluish-green with pale yellow marbling in *R. helenae*), and black webbing with blue veins and yellowish orange margins (vs. webbing proximally black and distally greenish and margins of webbing pale green or yellow in *R. helenae*).

Range

Known from the type locality, Camel Trophy of the Maliau Basin Conservation Area, Sandakan Division, State of Sabah, Malaysian Borneo; Batang Ai, State of Sarawak, Malaysian Borneo (Chan and Grismer, 2010).

Natural history

In Camel Trophy, an amplexant pair of the type specimens were found at night perching on a tree branch (<1 m) extending above the surface of a shallow pool (3–5 m × 10 m). The air temperature before the time of finding was 24°C. No tadpoles or eggs were found in the pond and calling males were absent in early March. Frogs found associated with *R. borneensis* included *Kurixalus appendiculatus* (Günther, 1858), *Polypedates macrotis* (Boulenger, 1891), *Chiromantis* sp., and *M. petrigena* Inger and Frogner, 1979.

DISCUSSION

Because of limited data available from GenBank, phylogenetic relationships were poorly

resolved in the tree constructed. However, the result suggested many interesting taxonomic problems related to GenBank data of *R. reinwardtii*. In the tree, *R. borneensis* formed a clade with the sequence of the sample from Malaysia (GQ204713: Meegaskumbura et al. [2010]) kept in the Field Museum of Natural History (FMNH235034), which was collected from Sipitang District, Sabah, according to the museum catalogue. In addition to the very small genetic distances (0–0.2%), geographical proximity of the frogs strongly suggests their conspecific status. *Rhacophorus reinwardtii* from Sarawak (JN377364–377366; voucher of JN377366=Naturhistorisches Museum Bern; Switzerland (NMBE) 1056517 from Batang Ai National Park, Sarawak: Hertwig et al., 2012) also differed from *R. borneensis* with very small genetic distances (0.2–0.8%), and was strongly suggested to be conspecific with the new species. These altogether are heterospecific with topotypic Javanese *R. reinwardtii* (GQ204720 [voucher=Zoological Reference Collection, Singapore, ZRC1.1.5273: Meegaskumbura et al., 2010]) with larger genetic distances (4.6–5.2%).

Interestingly, the reported sequence of *R. reinwardtii* from China (AF458146: Wilkinson et al., 2002) was not similar to the others examined in this study, with large distances of 8.2–11.2% between them. This sequence is from a frog stored at the National Museum of Natural Sciences, Taichung as NMNS3213 (Wilkinson et al., 2002), and is from China (Wilkinson et al., 2005). The sequence is actually very similar (distance of 0.8%) to that of *R. rhodopus* Liu and Hu, 1960 from Mengyang, Jinghong, China (EU215531: Li et al., 2008, 2009), although the sequence of *R. rhodopus* itself seems to be variable (cf. Li et al., 2012). *Rhacophorus reinwardtii* previously reported from China (e.g., Liu and Hu, 1961; Fei, 1999) are now assigned to *R. kio* (e.g., Fei et al., 2009), and our DNA analyses supported such a classification for other Chinese samples (EU215532, EF564570, EF564571, EF646371, EF646372, all from Yunnan [Li et al., 2008; Yu et al., 2007, 2008]). Thus there

seems to be little possibility of the presence of another cryptic species related to *R. reinwardtii* in China. We consider the species identification of the sequence AF458146 to be incorrect. Similarly, the sequence of *R. reinwardtii* from an unknown locality, but probably from Vietnam (AF285225: Thomas Ziegler, unpublished data), outgrouped all the above taxa studied with very large genetic distances (9.5–12.2%). However, the sequence is completely identical with that of *R. calcaneus* Smith, 1924 from Lao (GQ204719: Meegaskumbura et al., 2010), and only slightly differs (0.2%) from the sequence of *R. orlovi* Ziegler and Köhler, 2001 from Vietnam (DQ283380: Frost et al., 2006). Thus, some sequences currently used as that of *R. reinwardtii* require great caution to be used as references in the systematic studies. Because *R. reinwardtii* and its allies are so unique morphologically, voucher specimens would not have been misidentified; incorrect identifications may have arisen from mistakes in handling tissue samples.

Recent description of new species that were split from *R. reinwardtii* heavily relied on the body color (Ohler and Delorme, 2006; Chan and Grismer, 2010; Rowley et al., 2012) as is the case in the present new species. However, there are indeed intraspecific variations as briefly noted for *R. helenae* by Rowley et al. (2012). Although the type specimens of *R. borneensis* were plain green on the dorsum, other specimens may have marking. In their field guide of Bornean frogs, Inger and Stuebing (1997) showed a photograph of *R. reinwardtii* having the dark green dorsum scattered with dark spots, and posterior flank and anterior face of the thigh with black and blue spots. Unfortunately, Inger and Stuebing (1997) did not indicate the locality where the photograph was taken, but they described the body size of males (46–55 mm) and females (56–65 mm), which values match *R. borneensis*, although their description of the ventral color to be pure white contrasts with the yellowish orange in *R. borneensis*. In the original description of *R. norhayatii* from the continent, Chan and Grismer (2010) noted that Bornean *R. rein-*

wardtii has faint, white spots on the dorsum interspersed with the dark spots, which are not observed in Javanese frogs, and suggested that Bornean *R. reinwardtii* might be a separate lineage on its own based on this, together with body size difference, although Chan and Grismer (2010) did not show the body size or voucher number of Bornean *R. reinwardtii*. From the results of our phylogenetic analysis, we think at present that there is little possibility of the presence of multiple species related to *R. reinwardtii* in Borneo. Rather, we think color pattern differences like the occurrence of spots on the dorsum and ventral coloration are within intraspecific variations, possibly found in additional specimens of *R. borneensis*.

Although the gliding frogs are conspicuous because of their relatively large size, vivid coloration, and enormously broad webs, they usually live on tall trees and can not easily be sampled. They can be encountered only by chance, mostly in the breeding seasons, when the frogs come down from the canopy to aggregate on low vegetation around the pool. However, logging of primary forests is still ongoing on the island of Borneo, leading to the extinction of forest species like gliding frogs before their actual diversity is unveiled.

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LITERATURE CITED

- BORDOLOI, S., BORTAMULI, T., AND OHLER, A. 2007. Systematics of the genus *Rhacophorus* (Amphibia, Anura): identity of red-webbed forms and description of a new species from Assam. *Zootaxa* 1653: 1–20.
- BROWN, W. C. AND ALCALA, A. C. 1994. Philippine frogs of the family Rhacophoridae. *Proceedings of the California Academy of Sciences* 48: 185–220.
- CHAN, K.-O. AND GRISMER, L. L. 2010. Re-assessment of the Reinwardt's Gliding Frog, *Rhacophorus reinwardtii* (Schlegel 1840) (Anura: Rhacophoridae) in Southern Thailand and Peninsular Malaysia and its re-description as a new species. *Zootaxa* 2505: 40–50.
- DUBOIS, A. 1987 (1986). *Miscellanea taxinomica [sic] batrachologica* (I). *Alytes* 5: 7–95.
- FEI, L. (Ed.). 1999. *Atlas of Amphibians of China*. Henan Science and Technology Press, Zhengzhou.
- FEI, L., HU, S.-Q., YE, C.-Y., AND HUANG, Y.-Z. 2009. *Fauna Sinica. Amphibia. Volume 2. Anura*. Chinese Academy of Science. Science Press, Beijing.
- FROST, D. R. 1985. *Amphibian Species of the World: A Taxonomic and Geographical Reference*. Allen Press, Lawrence.
- FROST, D. R. 2013. *Amphibian Species of the World: an Online Reference. Version 5.6 (9 January, 2013)*. American Museum of Natural History, New York. <http://research.amnh.org/herpetology/amphibia/index.html>
- FROST, D. R., GRANT, T., FAIVOVICH, J. N., BAIN, R. H., HAAS, A., HADDAD, C. F. B., DE SÁ, R. O., CHANNING, A., WILKINSON, M., DONNELLAN, S. C., RAXWORTHY, C. J., CAMPBELL, J. A., BLOTTO, B. L., MOLER, P., DREWES, R. C., NUSSBAUM, R. A., LYNCH, J. D., GREEN, D. M., AND WHEELER, W. C. 2006. The amphibian tree of life. *Bulletin of the American Museum of Natu-*

- ral History* 297: 1–370.
- HARVEY, M. B., PEMBERTON, A. J., AND SMITH, E. N. 2002. New and poorly known parachuting frogs (Rhacophoridae: *Rhacophorus*) from Sumatra and Java. *Herpetological Monographs* 16: 46–92.
- HERTWIG, S., DAS, I., SCHWEIZER, M., BROWN, R. M., AND HAAS, A. 2012. Phylogenetic relationships of the *Rhacophorus everetti*-group and implications for the evolution of reproductive modes in *Philautus* (Amphibia: Anura: Rhacophoridae). *Zoologica Scripta* 41: 29–46.
- INGER, R. F. 1966. The systematics and zoogeography of the Amphibia of Borneo. *Fieldiana: Zoology* 52: 1–402.
- INGER, R. F. AND STUEBING, R. B. 1997. *A Field Guide to the Frogs of Borneo*. Natural History Publications, Kota Kinabalu, Sabah.
- INGER, R. F., STUART, B. L., AND ISKANDAR, D. T. 2008. Systematics of a widespread Southeast Asian frog, *Rana chalconota* (Amphibia: Anura: Ranidae). *Zoological Journal of the Linnean Society* 155: 123–147.
- LI, J.-T., CHE, J., BAIN, R. H., ZHAO, E.-M., AND ZHANG, Y.-P. 2008. Molecular phylogeny of Rhacophoridae (Anura): a framework of taxonomic reassignment of species within the genera *Aquixalus*, *Chiromantis*, *Rhacophorus*, and *Philautus*. *Molecular Phylogenetics and Evolution* 48: 302–312.
- LI, J.-T., CHE, J., MURPHY, R. W., ZHAO, H., ZHAO, E.-M., RAO, D.-Q., AND ZHANG, Y.-P. 2009. Evolution of reproduction in the Rhacophoridae (Amphibia: Anura) inferred from a phylogenetic analysis of five nuclear and three mitochondrial genes. *Molecular Phylogenetics and Evolution* 53: 509–522.
- LI, J.-T., LI, Y., MURPHY, R. W., RAO, D.-Q., AND ZHANG, Y.-P. 2012. Phylogenetic resolution and systematics of the Asian tree frogs, *Rhacophorus* (Rhacophoridae, Amphibia). *Zoologica Scripta* 41: 557–570.
- LIEM, S. S. 1970. The morphology, systematics and evolution of the Old World tree frogs (Rhacophoridae and Hyperoliidae). *Fieldiana: Zoology* 57: 1–145.
- LIU, C.-C. AND HU, S.-C. 1961. *Chinese Tailless Batrachians*. Ka-sue-chu-ban-sha, Beijing.
- MATSUI, M. 1984. Morphometric variation analyses and revision of the Japanese toads (genus *Bufo*, Bufonidae). *Contributions from the Biological Laboratory, Kyoto University* 26: 209–428.
- MATSUI, M. 1994. A taxonomic study of the *Rana narina* complex, with description of three new species (Amphibia: Ranidae). *Zoological Journal of the Linnean Society* 111: 385–415.
- MATSUI, M. AND PANHA, S. 2006. A new species of *Rhacophorus* from eastern Thailand (Anura: Rhacophoridae). *Zoological Science* 23: 477–481.
- MATSUI, M., HAMIDY, A., BELABUT, D. M., AHMAD, N., PANHA, S., SUDIN, A., KHONSUE, W., OH, H.-S., YOUNG, H.-S., JIANG, J.-P., AND NISHIKAWA, K. 2011. Systematic relationships of oriental tiny frogs of the family Microhylidae (Amphibia, Anura) as revealed by mtDNA genealogy. *Molecular Phylogenetics and Evolution* 61: 167–176.
- MEEGASKUMBURA, M., MEEGASKUMBURA, S., BOWATTE, G., MANAMENDRA-ARACHCHI, K., PETHIYAGODA, R., HANKEN, J., AND SCHNEIDER, C. J. 2010. *Taruga* (Anura: Rhacophoridae), a new genus of foam-nesting tree frogs endemic to Sri Lanka. *Ceylon Journal of Science (Biological Science)* 39: 75–94.
- OHLER, A. AND DELORME, M. 2006. Well known does not mean well studied: morphological and molecular support for existence of sibling species in the Javanese gliding frog *Rhacophorus reinwardtii* (Amphibia, Anura). *Comptes Rendus Biologies, Paris* 329: 86–97.
- OHLER, A. AND DUBOIS, A. 2006. *Hyla reinwardtii* Schlegel, 1840 as a nomen protectum. *Alytes* 23: 123–132.
- ROWLEY, J. J. L., TRAN, D. T. A., HOANG, H. D., AND LE, D. T. T. 2012. A new species of large flying frog (Rhacophoridae: *Rhacophorus*) from lowland forests in southern Vietnam. *Journal of Herpetology* 46: 480–487.
- SHIMADA, T., MATSUI, M., YAMBUN, P., AND SUDIN, A. 2011. A taxonomic study of Whitehead's torrent frog, *Meristogenys whiteheadi*, with descriptions of two new species (Amphibia: Ranidae). *Zoological Journal of the Linnean Society* 161: 157–183.

- STUART, B. L., INGER, R. F., AND VORIS, H. K. 2006. High level of cryptic species diversity revealed by sympatric lineages of Southeast Asian forest frogs. *Biology Letters* 2: 470–474.
- WILKINSON, J. A., DREWES, R. C., AND TATUM, O. L. 2002. A molecular phylogenetic analysis of the family Rhacophoridae with an emphasis on the Asian and African genera. *Molecular Phylogenetics and Evolution* 24: 265–273.
- WILKINSON, J. A., THIN, T., LWIN, K. S., AND SHEIN, A. K. 2005. A new species of *Rhacophorus* (Anura: Rhacophoridae) from Myanmar (Burma). *Proceedings of the California Academy of Sciences* 56: 42–52.
- YU, G.-H., RAO, D.-Q., YANG, J.-X., AND ZHANG, M.-W. 2007. Non-monophyly of *Rhacophorus rhodopus*, *Theloderma*, and *Philautus albopunctatus* inferred from mitochondrial 16S rRNA gene sequences. *Zoological Research* 28: 437–442.
- YU, G.-H., RAO, D.-Q., YANG, J.-X., AND MANG, M. 2008. Phylogenetic relationships among Rhacophorinae (Rhacophoridae, Anura, Amphibia), with an emphasis on the Chinese species. *Zoological Journal of the Linnean Society* 153: 733–749.
- YU, G.-H., ZHANG, M.-W., AND YANG, J.-X. 2013. Molecular evidence for taxonomy of *Rhacophorus appendiculatus* and *Kurixalus* species from northern Vietnam, with comments on systematics of *Kurixalus* and *Gracixalus* (Anura: Rhacophoridae). *Biochemical Systematics and Ecology* 47: 31–37.

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